THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
The Business of Innovation

TECHNOLOGY TYPE:	**MICROCYSTIN TEST KIT**	
APPLICATION:	**RECREATIONAL WATER MICROCYSTIN DETECTION**	
TECHNOLOGY NAME:	**Microcystin Strip Test Kit**	
COMPANY:	**Abraxis**	
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ETV Joint Verification Statement

The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of microcystin test kits for water monitoring. This verification statement provides a summary of the test results for the Abraxis Microcystin Strip Test Kit.

VERIFICATION TEST DESCRIPTION

This verification test of the Abraxis Microcystin Strip Test Kit was conducted from July 26 through August 12, 2010 at Battelle laboratories in Columbus, OH. Reference analyses by liquid chromatography tandem mass

spectrometry (LC-MS/MS) were performed the week of August 16, 2010 by the University of Nebraska Water Sciences Laboratory.

The objective of this verification test was to evaluate the microcystin test kit performance in analyzing known concentrations of microcystin in ASTM International Type II deionized (DI) water and in natural recreational water (RW) samples. The technology was used to analyze a variety of water samples for the variants microcystin-LR, microcystin-LA, and microcystin-RR. Because the technology cannot specify between the more than 80 microcystin variants, the samples prepared for this test were spiked with three individual variants. The Microcystin Strip Test Kit provided a quantitative determination of microcystins and was evaluated in terms of:

- Accuracy - comparison of test kit results (samples prepared in DI) to results from a reference method;
- Precision - repeatability of test kit results from three sample replicates analyzed in DI water, matrix interference, and RW samples;
- Linearity - determination of whether or not the test kit response increases in direct proportion to the known concentration of microcystin;
- Method detection limit - the lowest quantity of toxin that can be distinguished from the absence of that toxin (a blank value) at a 95% confidence level;
- Inter-kit lot reproducibility - determination of whether or not the test kit response is significantly different between two different lots of calibration standards within the kits;
- Matrix Interference - evaluation of the effect of natural recreational water matrices and chlorophyll-*a* on the results of the test kits; and
- Operational and sustainability factors - general operation, data acquisition, setup, consumables, etc.

Each microcystin test kit was operated according to the vendor's instructions by a vendor-trained Battelle technician. Samples and calibration standards were analyzed in duplicate and positive and negative controls were analyzed at the vendor-specified frequency. The ability of the Abraxis Microcystin Strip Test Kit to determine the concentration of microcystin was challenged using quality control (QC) samples, performance test (PT) samples and RW samples. QC, PT, and RW samples were prepared by Battelle technical staff the day before testing began. The test samples were prepared in glass volumetric flasks and stored in amber glass vials at 4 °C ± 3 °C until use. The reference samples that were prepared from the test solutions were stored in amber glass bottles at < -10°C. Replicate samples for the test kits were taken from the same sample bottle. The QC, PT, and RW samples were prepared blindly for the operator by coding the sample labels to ensure the results were not influenced by the operator's knowledge of the sample concentration and variant.

Unlike many contaminants, certified microcystin standards are not commercially available. In planning this verification test, multiple sources of standards were investigated. With agreement from the stakeholders, all vendors and the EPA project officer, the standards used for this verification were purchased from the most reputable sources (LR and RR from Canadian National Research Council and LA from Abraxis), based on a Performance Evaluation Audit, and used for both the testing solutions and the reference method calibration.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted technical systems audits of the both the laboratory and field testing, and Battelle QA staff conducted a data quality audit of at least 10% of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

Following is a description of the Abraxis Microcystin StripTest Kit, based on information provided by the vendor. The information provided below was not verified in this test.

The Microcystin Strip Test Kit is a rapid immunochromatographic test, designed solely for use in the qualitative screening of microcystins and nodularins in RW. A rapid cell lysis step (QuikLyse™) performed prior to testing is required to measure total microcystins (dissolved or free, plus cell bound). The Microcystin Strip Test Kit provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA,

high-pressure liquid chromatography (HPLC) or other conventional methods. The test is designed for field use, requiring no instrumentation or other equipment, no power sources, and no refrigerated storage.

The test is based on the recognition of microcystins, nodularins and their variants by specific antibodies. The toxin conjugate competes for antibody binding sites with microcystins/nodularins that may be present in the water sample. The test device consists of a conical flip-top vial with specific antibodies for microcystins and nodularins labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a non-microcystin antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of microcystins in the water sample, and therefore, it should be present in all reactions. In the absence of toxin in the water sample, the colloidal gold labeled antibody complex moves with the water sample by capillary action to contact the immobilized microcystins conjugate producing a visible line in the test line region.

If microcystins are present in the water sample, they compete with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of toxin is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate and preventing the development of a second line in the test line region. If a second line is not visible in the test line region, or if the test line is lighter than the negative control line, microcystin is present at levels of concern (>10 ppb). Semi-quantitative results in the range of 0 to 10 ppb can be obtained by comparing the test line intensity to those produced by solutions of known microcystins concentrations (control solutions). During this verification, the results were considered 0 to 10 ppb, or greater than 10 ppb.

VERIFICATION RESULTS

The verification of the Abraxis Microcystin Strip Test Kit is summarized in Table 1 and by the parameters described below.

Table 1. Abraxis Microcystin Strip Test Kit Performance Summary

Sample Description	LR Results		LA Results		RR Results	
Spiked Conc.	(Observation and Interpretation)		(Observation and Interpretation)		(Observation and Interpretation)	
0.10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb
	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb
	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb	Dark Line	0 – 10 ppb
0.50 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
1.0 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
2.0 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
	Line	0 – 10 ppb	Line	0 – 10 ppb	Line	0 – 10 ppb
4.0 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb
	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb
	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb	Faint line	0 – 10 ppb
7.0 ppb	NA	NA	Very Faint line	0 – 10 ppb	No line	> 10 ppb
	NA	NA	Very Faint line	0 – 10 ppb	No line	> 10 ppb
	NA	NA	Very Faint line	0 – 10 ppb	No line	> 10 ppb
15 ppb	No line	> 10 ppb	No line	> 10 ppb	No line	> 10 ppb
	No line	> 10 ppb	No line	> 10 ppb	No line	> 10 ppb
	No line	> 10 ppb	No line	> 10 ppb	No line	> 10 ppb

NA = not applicable; 7.0 ppb level was not performed for LR samples.

Accuracy. The DI samples (concentrations between 0.10 and 15 ppb) for the three variants of microcystin were used during this verification test. All of the samples were analyzed in triplicate and each of them produced a

control line on the Microcystin Strip Test Kit to indicate that the test was functioning properly. As the concentrations of the various microcystin variants increased, the line generated in the test area region was observed to change as expected. The lowest concentration samples generated dark lines, the mid-level concentrations generated lighter lines, and the highest concentrations were either very faint lines or generated no line at all. The Microcystin Strip Test Kit correctly detected each of the 15 ppb samples as being greater than 10 ppb. The Microcystin Strip Test Kit results were inconsistent with the reference method in only one sample set out of 18. The 7.0 ppb RR DI water sample did not generate a line, indicating a concentration of greater than 10 ppb.

Precision. The line colors were consistent within the three replicates at each concentration, with one exception. With the exception of RW 5, the triplicate measurements of the samples generated lines that were very similar in intensity. In two replicates, RW 5 generated faint lines indicating a 0 to 10 ppb concentration and one replicate that had no line, indicating a concentration of greater than 10 ppb.

Matrix Interference. The RW matrix interferent and chlorophyll-*a* interferent sample results for the Microcystin Strip Test Kit all agreed and the interpretation of the results was consistent with the spiked amount of microcystins in the samples. There was no indication that different matrices affected the test kit performance.

Recreational Water (RW). RW sample results for the Microcystin Strip Test Kit were also consistent with the reference method results. While the total measured microcystin result may have other variants present that were not measured by the reference method, with the exception of RW 5, the sample results were all consistent with the reference laboratory results of the RW samples. That is, when the reference concentrations were greater than 10 ppb, there was no line generated; when the reference concentration was between 2.0 and 4.0 ppb, the lines were medium dark or faint, and when the reference concentrations were lower than 2.0 ppb, the lines generated were dark.

Operational Factors. The test kit operator reported that the Microcystin Strip Test Kit was very easy to use and needs no technical skills to operate. The brochure and flow charts with illustrations were clear and easy to follow. There is no solution or sample preparation needed. The entire procedure takes approximately 40 minutes, including the QuikLyse™ procedure and the microcystins analysis. The QuikLyse™ process uses 1 mL of sample through two, 8-minute incubation periods. The sample is then transferred into the microcystins reagent conical tube. The sample is incubated for 10 minutes and then the test strip is added to the conical tube. The test strip is interpreted according to the figure in the brochure after 5 minutes of exposure to the sample. No consumables were required for this technology. Once the analysis was complete, the remaining solutions were disposed in the trash in accordance with local regulations. The listed price for the Microcystin Strip Test Kit at the time of the verification test was $480 for a 20-strip kit and $150 for a five-strip kit. The kit has a 12-month shelf life when received, and should be stored at room temperature.

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